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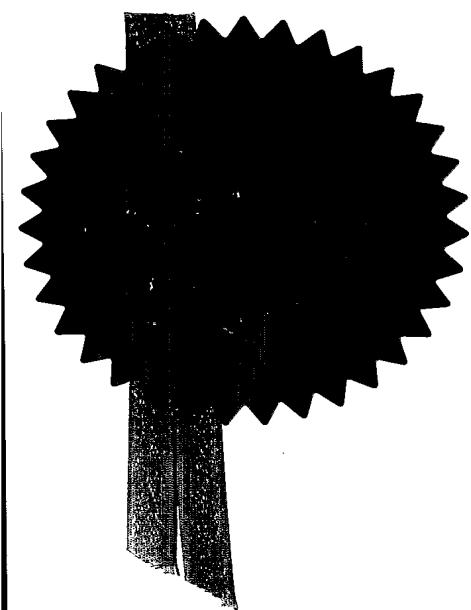
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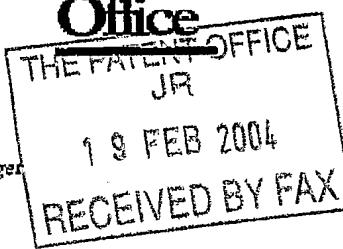


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If the applicant is a corporate body, give the country/state of its incorporation

7409436004

4. Title of the invention

METHODS

5. Name of your agent (if you have one)

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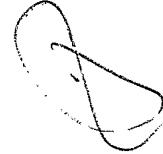
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Description 39

Claims(s) 3

Abstract 1

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Date

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Methods

The present invention relates to pain relief and, in particular, compounds for use as analgesics.

5

The control and relief of pain is an important branch of medicine. Pain may come about both as a result of disease or injury as well as a result of medical treatment such as chemotherapy. In each case, it is important to alleviate the pain as much as possible so as to enable the sufferer to function 10 normally.

Pain is a complex sensation and is the most common symptom of disease. Pain may be nociceptive pain, which is due to a noxious stimulus (chemical, thermal, mechanical etc.) that activates pain receptors, or neuropathic pain, 15 which results from dysfunction of the central or peripheral nervous system and is often poorly localised. Pain may also be classified as being acute or chronic in nature. Acute pain is usually a result of injury (e.g. trauma or disease), it lasts a short time and is typically resolves as the injured tissue heals or soon after. Chronic pain is usually defined broadly and arbitrarily 20 as a pain persisting for over one month beyond the resolution of an acute tissue injury, pain persisting or recurring for more than three months, or pain associated with tissue injury that is continued or progressed (The Merck Manual, 1999).

25 Several pain syndromes are difficult to classify according to these criteria. These include, for example, chronic headache and continuous acute pain produced by the invasion of body tissues in malignant diseases.

30 Two neural pathways relating to pain act concurrently in the body: (1) a sensory pathway which senses tissue damage and subsequently produces a

feeling of pain; (2) an analgesic pathway which reduces the feeling of pain and prevents the flow of information about the pain to the central nervous system (CNS), thus allowing the organism to maintain its normal activity in spite of an injury. Since these are different pathways, they are affected by 5 different substances. For example, aspirin and lidocaine are active on the peripheral sensory pathway, while morphine and related substances are active on the analgesic system.

Opioids, such as morphine and related opioid compounds are considered as 10 the most potent analgesic agents and are often required for relief of severe pain. However, these narcotic drugs have the severe drawback of leading to dependence and addiction. In addition, patients treated with opioids tend to develop tolerance to the drug, which leads to increasing dosage of the drug being needed for exerting the analgesic effect and to subsequent withdrawal 15 symptoms. Further side effects associated with opioid drugs include nausea, sedation and respiratory depression.

Nonopioid analgesics, e.g. cyclooxygenase inhibitors such as 20 acetaminophen (paracetamol) and other nonsteroidal anti-inflammatory drugs (NSAIDs) are often effective for treatment of mild to moderate pain. Anti-inflammatory agents of the NSAID class such as acetylsalicylic acid (aspirin), indomethacin, diclofenac and benzylamine have been used as analgesics in pain associated with trauma and inflammation. Common side effects of the NSAID class of drugs include gastrointestinal irritation and 25 ulceration, blockade of platelet aggregation, renal dysfunction and hepatic damage.

Another major class of analgesics is the local anaesthetics that block sodium channels. Compounds of this class, e.g. lidocaine, when topically applied to 30 the spine, have been found effective for control of pain after surgery or

trauma, but require expertise and infrastructure to administer and monitor properly. Systemic infusion of lidocaine can reduce acute pain, but requires continuous monitoring so that resuscitation from seizures or apnea can be performed immediately.

5

Additional classes of analgesics include local anaesthetics, NMDA receptor antagonists, antidepressants and anticonvulsants.

In addition to the above classes of analgesics, strong laboratory evidence now underwrites anecdotal claims of cannabinoid analgesia in inflammatory and neuropathic pain (Rice et al (2003) *Cannabinoids and pain*. In: Dostrovsky, Carr and Kolzenburg Eds. Seattle: IASP Press, 437-468). Recent advances have dramatically increased our understanding of cannabinoid pharmacology: the psychoactive constituents of *Cannabis sativa* have been isolated, synthetic cannabinoids described and an endocannabinoid system identified, together with its component receptors, ligands and their biochemistry. Sites of analgesic action have been identified in brain, spinal cord and the periphery, with the latter two presenting attractive targets for divorcing the analgesic and psychotropic effects of cannabinoids.

To date two cannabinoid receptors have been cloned, CB₁ in 1990 (Matsuda et al (1990) *Nature* 346, 561-564) and CB₂ in 1993 (Munro et al (1993) *Nature* 365 61-65). Both are G-protein coupled receptors and possess the typical structure consisting of an extracellular N-terminal domain, seven transmembrane helices and an intracellular C-terminal domain.

Several endogenous ligands that bind to CB₁ receptors have been discovered: the two prototypical ligands are anandamide (arachidonyl ethanolamide, AEA) and 2-arachidonylglycerol (2-AG), both

derivatives of a long chain fatty acid (Figure 1). AEA binds to the CB₁ receptor and evokes the classical tetrad of cannabinoid effects, ie analgesia, hypomotility, catalepsy and hypothermia, as well as having effects on memory processes, spasticity and cell proliferation. It also binds to the CB₂ receptor, where it does not evoke biologically significant activity, and is a weak agonist at the vanilloid receptor (VR1). 2-AG also has cannabimimetic effects and is found endogenously at much higher concentrations than AEA. It binds only weakly to CB₁, but is a full agonist, and also binds to CB₂ receptors. It has been suggested that 2-AG is the 5 endogenous ligand at the CB₂ receptor (Hillard (2000) Prostaglandins Other 10 Lipid Mediat 61, 3-18).

Palmitoylethanolamide (PEA) (Figure 1) is another endogenous cannabimimetic compound that is a fatty acid derivative. It possesses only 15 limited binding at CB₁ or CB₂ receptors, yet some of its effects are prevented by the CB₂ receptor antagonist SR144528 (SR2) (Calignano et al (1998) Nature 394 277-281). PEA has anti-inflammatory and analgesic effects *in vivo* (Calignano et al (1998) Nature 394 277-281; Jaggar et al (1998) Pain 76 189-199; Jaggar et al (1998) Neuroscience Letters 253 123- 20 126; Lambert et al (2001) Epilepsia 42 321-327), and there are several theories on how this effect is mediated: PEA may be enhancing the effects of other endogenous ligands that bind to the CB₂ receptor (the entourage effect); or it may be binding to a new non-CB₁/CB₂ receptor at which SR2 25 also acts as an antagonist.

Fatty acid amide hydrolase (FAAH) is an enzyme that degrades many fatty acid amides including the endocannabinoids (Boger et al (2000) Bioorganic and Medicinal Chemistry Letters 10 2613-2616). It is distributed widely in the brain (Egertova et al (1998) Proc R Soc Lond 265 2081-2085; Tsou et al 30 (1998) Neuroscience Letters 254 137-140; Romero et al (2002) Molecular

Brain Res 100 85-93) and periphery and is known to degrade both AEA and PEA (Tiger et al (2000) Biochemical Pharmacology 59 647-653). Recent evidence has shown that FAAH has complementary localisation with CB₁ receptors in many brain regions (Egertova et al (1998) Proc R Soc Lond 265 5 2081-2085) including those involved in pain pathways.

As FAAH degrades fatty acid amides, including the endocannabinoids AEA and PEA, inhibiting FAAH function *in vivo* may have the effect that the endocannabinoids persist for longer and thus may have more prolonged 10 effects, for example analgesic effects. Towards these goals various FAAH inhibitors have been discovered (Martin et al (2000) J Pharmacol Exp Ther 294 1209-1218; Boger et al (2000) Proc Natl Acad Sci USA 97 5044-5049) and, indeed, PEA itself has some ability to inhibit FAAH (Jonsson et al (2001) Br J Pharmacol 133 1263-1275).

15 Recently, a number of studies have been made of the structure-activity of PEA. Vandevoorde et al (2003) J Med Chem 46 1440-1448 synthesised analogues and homologues of PEA in which the ethanolamine head group was changed and found that none of the compounds were dramatically more 20 potent than PEA at reducing AEA metabolism by inhibiting FAAH function.

25 Similarly Jonsson et al (2001) Br J Pharmacol 13, 1263-1275 synthesised a number of analogues and homologues of PEA in which both the ethanolamine head group and the fatty acid tail were modified. The authors found that there was little difference in the ability of the analogues to reduce AEA metabolism by FAAH when compared to PEA. However, two of the compounds did increase AEA uptake into the cell when compared to PEA and so may be useful as 'entourage' compounds.

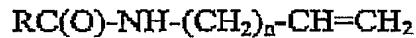
In a further study Lambert et al (1999) Biochem Biophys Acta 1440 266-274 examined the capacity of PEA and analogues and homologues having changes in both the ethanolamine head group and the fatty acid tail to act as ligands for CB₁ and CB₂. The authors found that PEA was a weak ligand for the CB₁ and CB₂ while the analogues and homologues essentially did not bind the receptors.

WO 00/32200 considers more anandamine analogues but does not provide any indication of activity *in vivo*.

10

We have found that compounds with similarities to PEA in which the ethanolamine group has been replaced by an allylamine group can act as an analgesic in animal models.

15 A first aspect of the invention provides the use of a compound of formula I:



wherein R represents C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl or C₂₋₂₀ alkynyl; and n is an integer from 0 to 3,

20

in the manufacture of a medicament for use in pain relief.

A second aspect of the invention provides a compound of formula I as hereinbefore defined for use in medicine.

25

A third aspect of the invention provides a method of providing pain relief to a patient comprising administering to said patient an effective amount of a compound of formula I as hereinbefore defined.

Alkyl, alkenyl and alkynyl groups referred to herein may be straight or branched-chain. For the avoidance of doubt, alkenyl groups may contain one or (where appropriate) more carbon-carbon double bonds (eg 1 to 3 C=C double bonds). Moreover, alkynyl groups may contain one or (where appropriate) more carbon-carbon triple bonds (eg 1 to 3 C≡C triple bonds). Preferably alkenyl and alkynyl groups contain only one point of unsaturation. Also preferably the alkyl, alkenyl or alkynyl group is straightchained.

10 The compounds used in the invention are amides of fatty acids having in common a terminal allylamine head group.

The patient is a patient having, or likely to have, a need for pain relief. By 'pain' we include all types of pain, for example acute and chronic pains, such as neuropathic pain and post-operative pain, chronic lower back pain, cluster headaches, herpes neuralgia, post herpetic neuralgia, phantom limb pain, central pain, dental pain, opioid-resistant pain, cancer-related pain, visceral pain, surgical pain, bone injury pain, pain during labour and delivery, pain resulting from burns, including sunburn, post partum pain, migraine, angina pain and genitourinary tract-related pain including cystitis. The term includes nociceptive pain or nociception.

15 20 25

The patient may be any animal in need of pain relief, for example a human, horse, pig, cow, sheep, chicken, dog, cat, rat or a mouse. Preferably the patient is a human patient.

An embodiment of the first or third aspects of the invention is wherein the medicament further comprises one or more analgesics or the patient is administered said further one or more analgesics.

The further analgesic is preferably a cannabinoid receptor ligand, for example arachidonylethanolamide (AEA) or 2-arachidonylglycerol (2-AG) or palmitolyethanolamide (PEA), or a substrate for FAAH, for example PEA. Examples of other analgesics are set out below.

5

An embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, R represents C_{10-20} alkyl, C_{10-20} alkenyl or C_{10-20} alkynyl.

10 A further embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, R represents C_{10-20} *n*-alkyl, C_{10-20} mono-alkenyl or C_{10-20} mono-alkynyl.

15 A further embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, R represents C_{10-20} *n*-alkyl, C_{10-20} mono-*n*-alkenyl or C_{10-20} mono-*n*-alkynyl.

20 A further embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, R represents C_{11-19} *n*-alkyl, or C_{11-19} mono-*n*-alkenyl.

A further embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, R represents C_{11-18} *n*-alkyl, or C_{11-18} mono-*n*-alkenyl.

25

A further embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, the alkenyl or alkynyl groups have no more than 3 C-C double or triple bonds, respectively.

A further embodiment of the first, second or third aspects of the invention is wherein the compound is not *N*-(2-propenyl) - 5,8,11,14-eicosatetraenamide.

5 A further embodiment of the first, second or third aspects of the invention is wherein, in the compound of formula I, n represents 0 or 1, preferably n represents 1.

An embodiment of the first, second or third aspects of the invention is 10 wherein the compound is *N*-(2-propenyl) hexadecanamide, *N*-(2-propenyl) cis-9-octadecenamide, *N*-(2-propenyl) cis-9-hexadecenamide, *N*-(2-propenyl) tetradecanamide, *N*-(2-propenyl) cis-9-tetradecenamide, *N*-(2-propenyl) octadecanamide, *N*-(2-propenyl) trans-9-octadecenamide, *N*-(2-propenyl) dodecanamide, or *N*-(2-propenyl) cis-5-dodecenamide. Preferably 15 the compound is *N*-(2-propenyl) hexadecanamide. Figure 1 includes illustrative diagrams of these compounds.

The compounds used in relation to the aspects of the invention may be 20 prepared by any suitable method, as would be appreciated by a person skilled in the art.

For example:

N-(2-propenyl) hexadecanamide may be prepared from palmitoleyl chloride 25 and allylamine as outlined in Scheme 1 of Vandevoorde et al (2003) J Med Chem 46 1440-1448.

N-(2-propenyl) cis-9-octadecenamide may be prepared from oleoyl chloride and allylamine. The compound is registered under CAS number 187529-39-30 1.

10

N-(2-propenyl) *cis*-9-hexadecenamide may be prepared from palmitoleic acid, oxalyl chloride and allylamine.

5 *N*-(2-propenyl) tetradecanamide may be prepared from myristoyl chloride and allylamine.

N-(2-propenyl) *cis*-9-tetradecenamide may be prepared from myristoleic acid, oxalyl chloride and allylamine.

10

N-(2-propenyl) octadecanamide may be prepared from stearoyl chloride and allylamine.

15

N-(2-propenyl) *trans*-9-octadecenamide may be prepared from elaidic acid, oxalyl chloride and allylamine.

N-(2-propenyl) dodecanamide may be prepared from lauroyl chloride and allylamine.

20

N-(2-propenyl) *cis*-5-dodecnamide may be prepared from *cis*-5-dodecenoic acid, oxalyl chloride and allylamine.

25

N-(2-propenyl) - 5,8,11,14-eicosatetraenamide may be prepared from arachidonic acid, oxalyl chloride and allylamine. The compound is registered under CAS number 177037-49-9. The compound is mentioned in Boger et al (1999) Bioorg Med Chem Lett 9, 1151-1154; Lin et al (1998) J Med Chem 41, 5353-5361; Pate et al (1996) Life Sci 58, 1849-1860; WO 00/32200.

The reagents mentioned above can be purchased from Sigma-Aldrich-Fluka, Acros Chimica. If the acyl chlorides are not commercially available, they might be prepared from the corresponding carboxylic acids and oxalyl chloride in dichloromethane as will be appreciated by a person skilled in the art.

Provided below is a detailed protocol for the synthesis of *N*-(2-Propenyl)hexadecanamide:

- 10 In a two neck flask, with 5.7 g (100 mmol) of allylamine was poured into 10 mL of dry methylene chloride. The solution was cooled in an ice bath and magnetically stirred. 2.74 g (10 mmol) of palmitoyl chloride was added dropwise. The reaction mixture was stirred for 12 h at room temperature and then washed with 5% sodium bicarbonate solution, 1 M HCl, and brine.
- 15 The organic layer was dried over MgSO₄, and after filtration, the solvent was evaporated under reduced pressure to give 1.74 g (59%) of a white solid:

Physical and spectral data: mp 61-63 °C (uncorrected);

- 20 TLC (ethyl acetate/methanol 8:2 v/v-1) *Rf* 0.77; 1H NMR (CDCl₃) % (ppm) 0.87 (t, *J*) 3 Hz, 3H), 1.22-1.54 (m, 26H), 2.19 (t, *J*) 7 Hz, 2H); 3.7-3.72 (m, 2H), 5.11-5.2 (m, 2H), 5.55 (NH), 5.79-5.88 (m, 1H); 13C NMR (CDCl₃) % (ppm) 14.10 (CH₃), 22.77, 25.88, 29.43, 29.56, 29.76, 32.02, 35.39, 25 36.87, 41.99, 58.48, 116.39 (CH₂), 134.51 (CH), 173.07 (C=O); mass spectrometry [M+•] 296; IR $\bar{\nu}$ (cm⁻¹) 3299 (NH), 1636 C=O). CAS number: 1012114-99-8.

The modifications required to synthesise further compounds used in the methods of the invention will be readily apparent to those of skill in the art.

The compounds of the invention will normally be administered orally or by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

10 Preferably, the formulation is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

In human therapy, the compounds of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

15 For example, the compounds of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The compounds of invention may also be administered *via* intracavernosal injection.

20 Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone,

25 hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC),

sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

5 Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or

10 dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intravenously, intra-arterially, epidurally, intraperitoneally, 15 intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be 20 suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

25 Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The 30 formulations may be presented in unit-dose or multi-dose containers, for

example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, 5 granules and tablets of the kind previously described.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention will usually be from 1 to 1000 mg per adult (*i.e.* from about 0.015 to 15 mg/kg), administered in single or 10 divided doses.

Thus, for example, the tablets or capsules of the compound of the invention may contain from 1 mg to 1000 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event 15 will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

20

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, *e.g.* 25 dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134ATM) or 1,1,1,2,3,3-heptafluoropropane (HFA 227EATM), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The 30 pressurised container, pump, spray or nebuliser may contain a solution or

suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a 5 powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains at least 1 mg of a compound of the 10 invention for delivery to the patient. It will be appreciated that the overall daily dose with an aerosol will vary from patient to patient, and may be administered in a single dose or, more usually, in divided doses throughout the day.

15 Alternatively, the compounds of the invention can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder. The compounds of the invention may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the 20 ocular route, particularly for treating diseases of the eye.

For ophthalmic use, the compounds of the invention can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in 25 combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the invention can be formulated as a suitable ointment containing the active compound 30 suspended or dissolved in, for example, a mixture with one or more of the

following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the

5 following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Formulations suitable for topical administration in the mouth include lozenges

10 comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

15 Generally, in humans, oral or topical administration of the compounds of the invention is the preferred route, being the most convenient. In circumstances where the recipient suffers from a swallowing disorder or from impairment of drug absorption after oral administration, the drug may be administered parenterally, e.g. sublingually or buccally.

20

For veterinary use, a compound of the invention is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular

25 animal.

Conveniently, the formulation is a pharmaceutical formulation.

30 Salts of the compounds to be used in the invention may be prepared in conventional manner, for example by reaction of the compound with an

appropriate base to form the corresponding base salt, or with an appropriate acid to form the corresponding acid salt. Physiologically acceptable acid salts include hydrochloride, sulphate, mesylate, besylate, phosphate and glutamate.

5 The compounds to be used in the invention may also be administered in the form of 'prodrugs'.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is capable of being enzymatically activated or converted into the more active parent form (see, for example, D.E.V. Wilman "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions* 14, 375-382 (615th Meeting, Belfast 1986) and V.J. Stella *et al* "Prodrugs: A Chemical Approach to Targeted Drug Delivery" *Directed Drug Delivery* R. Borchardt *et al* (ed.) pages 247-267 (Humana Press 1985)).

The prodrug may be, for example, easier to administer, more suitable for storage or less toxic or undesirable at the site of administration.

20 Several factors need to be taken into account in selecting an enzyme for prodrug activation. These include the molecular weight and physical properties of the enzyme, its activity and stability under physiological conditions, and the nature of the drug that the enzyme generates. The enzyme may be an endogenous enzyme or may be an exogenous enzyme targeted to a site where pain relief is required (for example a tumour site).

25 Enzymes of both mammalian and non-mammalian origin are currently being explored for the activation of a wide range of prodrugs (Senter *et al*, 1993. Generation of cytotoxic agents by targeted enzymes. *Bioconjugate 4*, 3-9; Senter *et al*, 1991. Activation of prodrugs by antibody-enzyme conjugates. *In*

Immunobiology of Proteins and Peptides VI, ed. M.Z. Atassi. Plenum Press, New York, pp 97-105). While enzymes of mammalian origin might be advantageous due to reduced immunogenicity, the prodrugs that they act upon might be substrates for corresponding endogenous enzymes.

5

Enzymes that may be useful in the method of this invention include, but are not limited to alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs, arylsulphatase useful for converting sulphate-containing prodrugs into free drugs, proteases, such as serratia protease, 10 thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs, D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents, carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs 15 into free drugs, β -lactamase useful for converting drugs derivatized with β -lactams into free drugs, and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art 20 as abzymes, can be used to convert the prodrugs of the invention into free active drugs [see, e.g. R.J. Massey, *Nature*, 328, pp. 457-458 (1987)].

Similarly, the prodrugs of this invention include, but are not limited to, the above-listed prodrugs, e.g., phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulphate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs. 25

A fourth aspect of the invention is a pharmaceutical composition comprising a compound as defined in the first aspect of the invention and one or more analgesics and a pharmaceutically acceptable excipient.

5 A fifth aspect of the invention is a kit of parts comprising:

- (a) a compound as defined in the first aspect of the invention; and,
- (b) one or more analgesics; and,
- (c) a pharmaceutically acceptable excipient

10

The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free. Means of administration of a pharmaceutical composition are outlined
15 above.

For example, the kit of parts may comprise a compound of formula I and one or more analgesics, wherein each are presented as a separate formulations and labelled for use together.

20

An embodiment of the first, third, fourth or fifth aspects of the invention is wherein the analgesic is an opioid, a non-steroidal anti-inflammatory drug, a local anaesthetic, a NMDA receptor antagonist, a cannabinoid, an antidepressant, and/or an anticonvulsant.

25

Examples of analgesics included in this embodiment of the aspects of the invention include opioid such as morphine, codeine, pethidine, methadone, aspirin and related compounds, ibuprofen, cyclooxygenase inhibitors such as acetaminophen (paracetamol), sodium channel blockers such as
30 lidocaine, dibucaine and tetracaine, calcium channel blockers, N-methyl-

D-aspartate (NDMA) receptor antagonists such as ketamine and phencyclidine, cannabinoids such as anandamide (arachidonylethanolamide, AEA) and 2-arachidonylglycerol (2-AG), antidepressants such as the tricyclic antidepressants imipramine and the serotonin re-take inhibitor 5 paroxetine, anti-convulsants such as gabapentin, carbamazepine and phenytoin and agents which interact with transient receptor potential (TRP) ion channels.

Any publications referred to herein are hereby incorporated by reference.

10

The invention will now be described in more detail by reference to the following non-limiting Figures and Examples.

Figure 1:

15 (A) The structure of anandamide (AEA), 2-arachidonylglycerol (2-AG), palmitoylethanolamide (PEA) and palmitoylallylamine (L-29).
(B) The structure of compounds with similarities to PEA

Figure 2: Depolarisation-induced suppression of excitation

20 An action potential at the pre-synaptic cell leads to neurotransmitter release and binding to the post-synaptic receptor. Subsequent calcium influx causes endocannabinoid synthesis and release. Endocannabinoids travel in a retrograde fashion across the synapse and bind to the pre-synaptic CB₁ receptor. This activates G-proteins which block calcium channels, making it 25 less likely that another action potential will reach threshold levels.

Figure 3:

30 (A) Anandamide and other cannabinoids are synthesised and released into the synapse, where they can bind to the CB₁ receptor. AEA re-enters the cell via its concentration gradient by facilitated diffusion or passive

diffusion. Once inside the cell endocannabinoids are broken down to inactive metabolites by FAAH.

(B) If FAAH is inhibited the concentration gradient into the cell is reduced and the amount of endocannabinoids present in the synapse increases,
5 thereby increasing binding to CB₁ receptors

Figure 4: Pain behaviour in the formalin test is attenuated by palmitoylallylamine.

(A) Time course of nociceptive activity (mean CPS-WST_(0,1,2) + S.E.M.) for
10 the control group, 0.1 mg/kg L-29, 1 mg/kg L-29 and 10 mg/kg L-29,
following s.c. injection of 2.5% formalin into the dorsal hind paw.

(B) Mean pain scores (mean CPS-WST_(0,1,2) + S.E.M.) for both phase 1 (0-
15 min) and phase 2 (15-60 min) of the formalin test for the control
group, 0.1 mg/kg L-29, 1 mg/kg L-29 and 10 mg/kg L-29.

15 * P<0.05 compared with control values by one-way ANOVA followed by
Dunnett's test

Figure 5: The effect of receptor antagonists on palmitoylallylamine mediated antinociception.

20 (A) Time course of nociceptive activity (mean CPS-WST_(0,1,2) + S.E.M.) for
the control group, 1 mg/kg L-29, and 1 mg/kg L-29 with prior
administration of either 1 mg/kg SR141716A, 1 mg/kg SR144528 or 10
mg/kg capsazepine.

(B) Mean pain scores (mean CPS-WST_(0,1,2) + S.E.M.) for both phase 1 (0-
25 15 min) and phase 2 (15-60 min) of the formalin test for the control
group, 1 mg/kg L-29, and 1 mg/kg L-29 with prior administration of
either 1 mg/kg SR141716A, 1 mg/kg SR144528 or 10 mg/kg
capsazepine.

P<0.05 compared with L-29 1 mg/kg values by one-way ANOVA
30 followed by Dunnett's test

Example 1: The Effect of Palmitoylallylamide, a Fatty Acid Amide Hydrolase Inhibitor, on Formalin-evoked Pain in the Rat.

Abstract

5 This study assessed the antinociceptive effect of a newly characterised fatty acid amide, palmitoylallylamide, in a rat model of inflammatory pain. Palmitoylallylamide is an analogue of palmitoylethanolamide, an endogenous cannabinomimetic compound. Palmitoylallylamide inhibits fatty acid amide hydrolase but does not significantly bind to either CB₁ or CB₂

10 receptors. The therapeutic effects of palmitoylallylamide on the behavioural response to subcutaneous formalin injection were tested. Control animals displayed the characteristic biphasic (phase 1 and phase 2) response to formalin injection. Palmitoylallylamide (10 mg/kg and 1 mg/kg i.p.) significantly alleviated pain behaviour compared to solvent control in

15 both phase 1 and phase 2. The selective CB₁ receptor antagonist, SR141716A (1 mg/kg i.p.) significantly attenuated the antinociception produced by palmitoylallylamide in phase 2 of the formalin test. The selective CB₂ antagonist SR144528 (1 mg/kg i.p.) and the selective VR1 antagonist capsazepine (10 mg/kg i.p.) did not reverse palmitoylallylamide

20 induced antinociception. These results support the hypothesis that fatty acid amide hydrolase inhibitors induce antinociception by increasing the extracellular levels of endocannabinoids and thereby increasing CB₁ receptor activation.

25 *Abbreviations:* L-29, Palmitoylallylamide; DMSO, Dimethyl sulphoxide; Δ⁹-THC, Δ⁹-tetrahydrocannabinol; PEA, Palmitoylethanolamide; SR1, SR141716A; SR2, SR144528; FAAH, Fatty acid amide hydrolase; i.p., Intraperitoneal; s.c., Subcutaneous; AEA, anandamide; 2-AG, 2-arachidonylglycerol; VR1, vanilloid receptor; CB₁, cannabinoid receptor 1;

30 CB₂, cannabinoid receptor 2, WIN2, WIN 55, 212-2;

1. Introduction

Cannabis has been used for thousands of years to provide hemp fibre and for its psychotropic and analgesic effects, but it wasn't until the 1960's that 5 meaningful research into cannabinoid compounds began. Since then the active constituents of cannabis have been described, synthetic cannabinoids have discovered and endogenous receptors and ligands identified.

The classical tetrad of cannabinoid effects are mediated by Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychoactive constituent of Cannabis sativa. These effects are analgesia, hypomotility, catalepsy and hypothermia. A major problem in cannabinoid research is how to separate the undesirable psychotropic effects of cannabinoids from their potential therapeutic benefits in pain, glaucoma, nausea and vomiting and spasticity. 10 15 The discovery of mammalian cannabinoid receptors and endogenous ligands is allowing greater understanding of the mechanisms behind the physiological effects of cannabinoid compounds.

To date two cannabinoid receptors have been cloned, CB₁ in 1990 (Matsuda 20 et al 1990) and CB₂ in 1993 (Munro et al 1993). CB₁ is expressed primarily on neurones. It has a very wide distribution in the brain, especially in pain processing areas (Egertova et al 1998) and is also found in the spinal cord (Farquhar-Smith et al 2000) and dorsal root ganglia. It appears to mediate most of the supraspinal effect of cannabinoids, as knockout mice (CB₁^{-/-}) do 25 not display the central effects of Δ^9 -THC (Ledent et al 1999). CB₂ receptors were originally found in splenic macrophages and seem to be restricted to immune cell lines, although there have been reports that show CB₂ in brain microglia. Activation of CB₂ receptors appears to have an anti-inflammatory effect; there is some evidence this may be due to down- 30 regulation of mast cells (Facci et al 1995).

Cannabinoids are unusual neurotransmitters as they are fatty acids, are synthesised 'on demand' instead of stored in the cell and usually travel in a retrograde fashion across the synapse. They appear to mediate the 5 phenomenon of depolarisation-induced suppression of excitation (DSE) in the brain. Post-synaptic depolarisation activates the enzymes that synthesise endocannabinoids in the post-synaptic cell. The newly synthesised endocannabinoids then diffuse out of the post-synaptic cell and travel backwards across the synapse to bind to CB₁ receptors on the pre- 10 synaptic cell membrane. Agonist binding to CB₁ receptors activates G-proteins that directly inhibit pre-synaptic Ca²⁺ entry into the pre-synaptic cell, thereby decreasing the probability of an action potential reaching threshold and neurotransmitter release (Krieter and Regehr 2001) (Figure 2). This may explain some of the analgesic properties of cannabinoids as 15 they may disinhibit the descending pain-modulating pathways from the midbrain periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) both of which contain CB₁ receptors (Egertova et al 1998).

Fatty acid amide hydrolase (FAAH) is an enzyme that degrades many fatty 20 acid amides including the endocannabinoids (Boger et al 2000a). It is distributed widely in the brain (Egertova et al 1998, Tsou et al 1998, Romero et al 2002) and periphery and is known to degrade both AEA and PEA (Tiger et al 2000). Recent evidence has shown that FAAH has complementary localisation with CB₁ receptors in many brain regions 25 (Egertova et al 1998) including those involved in pain pathways. After endocannabinoids have been released from the synapse, they re-enter the post-synaptic cell by passive diffusion and facilitated transport along their concentration gradient. AEA is thought to travel mainly by facilitated transport (Day et al 2001, Jacobsson and Fowler 2001) whereas PEA 30 transport is approximately 50% passive diffusion (Jacobsson and Fowler

2001) The two compounds may have different transporter molecules. Once inside the post-synaptic cell both compounds are metabolised by FAAH to inactive metabolites (Figure 3A) (Tiger et al 2000).

5 Mice lacking the gene that encodes for the enzyme FAAH (FAAH^{-/-}) have been developed (Cravatt et al 2001) which have reduced pain sensation and increased levels of anandamide, evidence that FAAH regulates the level of endogenous cannabinoids. Inhibiting FAAH should increase the levels of AEA (Martin et al 2000, Day et al 2001, Deutsch et al 2001) and PEA 10 inside the post-synaptic cell, thereby reducing the inward concentration gradient and therefore increasing the amount of endocannabinoids in the synapse. This will cause increased levels of CB₁ binding (Figure 3B). The same applies to peripheral FAAH and increased extracellular levels of endocannabinoid. There is evidence that inhibiting FAAH causes 15 cannabimimetic effects (Compton and Martin 1997), although the enzyme inhibitor used was not specific to FAAH.

Various FAAH inhibitors have been discovered (Martin et al 2000, Boger et al 2000b) and indeed PEA itself has some ability to inhibit FAAH (Jonsson et al 2001), evidence that lends weight to the entourage theory. The FAAH inhibitory compound that we are investigating is an analogue of PEA called palmitoylallylamine (L-29). It was developed by Dr Didier Lambert and team at the Unité de Chimie pharmaceutique et de Radiopharmacie, Université catholique de Louvain, Brussels, Belgium. The capacity of L-29 20 to inhibit FAAH was calculated by measuring the ability of the compound to inhibit FAAH catalysed [³H]-AEA hydrolysis (maximum inhibition and pI50 calculated). L-29 achieved 67% of the maximum inhibition ($\pm 3\%$), so was reasonably efficacious, with a pI50 of $5.47\mu\text{M} (\pm 0.06)$, so was quite 25 potent (unpublished data, but see (Jonsson et al 2001) for methods). In comparison, the values for PEA were 78% ($\pm 7\%$) and $5.3\mu\text{M} (\pm 0.15)$. 30

Binding to CB₁ and CB₂ receptors was also calculated by measuring the displacement of radioactive [³H]-CP55,940 from a cell line transfected with CB₁ receptors and radioactive [³H]-WIN55,212-2 from a cell line transfected with CB₂ receptors. At a concentration of 10µM, L-29

5 displaced 13.3% ($\pm 0.4\%$) in the CB₁ assay and 7.8% ($\pm 0.3\%$) in the CB₂ assay. For comparison PEA displaced 23.8% ($\pm 0.07\%$) and 13.9% ($\pm 1.7\%$) respectively, evidence that L-29 has a lower affinity at both the CB₁ and CB₂ receptors.

10 The formalin test was first described by Dubuisson and Dennis in 1977 (Dubuisson and Dennis 1977) and is a widely used and well-characterised model of acute and tonic inflammatory pain. Injection of dilute formalin into a rat hindpaw produces a biphasic pain-related behavioural response. There is an initial period of pain behaviour in the first five minutes after

15 injection, followed by a period of quiescence lasting about ten to fifteen minutes. This is followed by a second period of pain behaviour, which continues until the end of the experiment.

20 Many schemes of scoring this pain behaviour have been proposed. Dubuisson and Dennis describe a weighted score that measures the amount of time spent in each of three behavioural categories; favouring the affected paw, elevating the affected paw and licking the affected paw (Dubuisson and Dennis 1977). Others have counted the number of flinches of the affected limb. Two papers have attempted to validate the different scoring

25 methods: Abbott et al (1995) concluded that a simple sum of the time spent lifting and licking, or the scoring method of Dubuisson and Dennis were superior to any single measure and that adding flinching and favouring did not improve validity. Watson et al (1997) validated the optimal weighting method for the combined pain score and recommended alterations to the

30 Dubuisson and Dennis method. Favouring is not scored, elevating the paw

is given a weight of 1 and licking the paw is given a weight of 2. This new pain score was called the Combined Pain Score-Weighted Scores Technique (0,1,2) (CPS-WST_(0,1,2)) and is the method used in this study.

5 What are the mechanisms mediating the pain behaviour in the formalin test? It is generally believed that the first phase is due to direct chemical activation of primary afferent neurones of the C-fibre type (Puig and Sorkin 1995, Dalleil et al 1995, Mc Call et al 1996). It seems that the C-fibres at the site of formalin injection are destroyed by the injection and it is those that 10 are further from the injection site which receive a lower concentration of formalin that survive to respond to the stimulus (McCall et al 1996). Some authors believe that the second phase is due to the intense afferent barrage of the first phase causing central sensitisation in the dorsal horn neurones of the spinal cord (Martindale et al 2001). However, there have been 15 numerous electrophysiological experiments showing continued C-fibre activation, albeit at a reduced intensity, throughout the second phase (Puig and Sorkin 1995, Dalleil et al 1995); probably due to inflammation in the paw (Damas and Liegeois 1999). The most widely accepted hypothesis is that a combination of central sensitisation maintained by continuing primary 20 afferent firing, due to ongoing inflammation produces pain behaviour in the second phase. There is also evidence that the interphase is due to descending inhibition from higher centres to the spinal cord (Puig and Sorkin 1995, Henry et al 1999) however others have reported less firing of C-fibres in the interphase (McCall et al 1996).

25 The aims of this experiment were firstly, to determine if L-29 is antinociceptive in the formalin model of inflammatory pain, and then to determine where this effect is mediated. To do this we used specific receptor antagonists prior to giving L-29 to see if they would reverse any 30 possible antinociceptive effect. SR141716A (SR1) is a selective CB₁

receptor antagonist first described by (Rinaldi-Carmona et al 1995). Strangman et al (1998) described some of the pharmacokinetics of SR1. The authors administered WIN 55, 212-2 (WIN2), a potent CB₁ agonist, to rats inducing catalepsy. SR1 significantly antagonised WIN2 induced catalepsy within 15 min of administration (1 mg/kg i.p.), this effect lasted at least until the end of their experiments, approximately 50 minutes after administration. SR2 is a selective CB₂ receptor antagonist first described by (Ueda et al 2000). It has been used in studies at doses of 0.3 mg/kg and 3 mg/kg i.p. (Beaulieu et al 2000). Capsazepine is a competitive VR1 antagonist, developed by (Bevan et al 1991), and further described as antagonising the antinociceptive effect of capsaicin (Urban and Dray 1991, Dickenson and Dray 1991, Di Marzo et al 2001b). It has been used in studies at doses of 10 mg/kg i.p. (Bouaboula et al 1997). Therefore, we used SR1 (1 mg/kg), SR2 (1 mg/kg) and capsazepine (10 mg/kg) prior to L-29 administration.

2. Materials and Methods

2.1 Animals

All experiments conformed with British Home Office regulations. Male Wistar rats weighing 230-290g were obtained from B&K. Animals were housed in group cages and maintained on a 12-hour light-dark cycle with ad libitum access to food and water. Each animal was used in one experiment only.

2.2 Materials

L-29 was a gift from D Lambert (see Figure 1 for chemical structure). SR1 and SR2 were a gift from Sanofi and capsazepine was purchased from

Tocris. All drugs were dissolved in 40% dimethyl sulphoxide (DMSO) and saline.

2.3 Treatment groups

5 Rats were randomised into seven groups. Investigator JB carried out the dosing of the animals to conform with Home Office regulations, while investigator HJ carried out all other duties including scoring the behavioural test, she was blinded to the drugs given. All animals were acclimatised to the testing environment for at least 30 min prior to the experiment

10 beginning. Each animal received a first intraperitoneal (i.p.) injection of either vehicle or antagonist and five minutes later a second i.p. injection of either vehicle or L-29. All i.p. injections had a volume of 1 ml/kg. 10 min later all the animals received a formalin injection. See Table 1 for groups.

15 *2.4 Formalin Test*

50 μ l of 2.5% formalin was injected subcutaneously into the dorsum of the right hind paw via a 27G needle. The paw was then marked with permanent ink. The animal was immediately replaced in the observation chamber, a clear plexiglass box measuring 23cm x 18cm x 14 cm, with a mirror positioned underneath at 45° to allow an unobstructed view of the paw. Observations began as the rats were placed in the box and continued for 60 min. Their behaviour was rated according to the Composite Pain Score, Weighted Scores Technique (CPS-WST (0,1,2)), a validated pain behaviour scoring system for the rat formalin test (Watson et al 1997). The amount of time spent in each of 2 behavioural categories was measured for each consecutive 5 min period within the hour scored. Category 1, the injected paw is elevated and not in contact with any surface and category 2, the injected paw is licked, bitten or shaken. A weighted score was then obtained for each 5 min period and for phase 1 (0-15 min) and phase 2 (15-60 min), 30 by using the formula below:

$$\text{CPS-WST} = (1 \times \text{time in category 1}) + (2 \times \text{time spent in category 2})$$

Test session duration

5 2.5 Statistical Analysis

Data was analysed using the one-way ANOVA test followed by Dunnett's test using the SigmaStat 2.3 computer package.

10 3. Results

3.1 Effects of L-29 on formalin-evoked nociception

Figure 4A shows the time course of pain behaviour following formalin injection for the control group and all doses of L-29 and Figure 4B shows the mean pain score for phase 1 and phase 2 for these groups. The control group confirmed the previously described biphasic response to formalin (Dubuisson and Dennis 1977, Malmberg and Yaksh 1992, Abbott et al 1995) with an initial high pain score in the first 5 minutes, followed by a quiescent period and the beginning of the second phase, marked by increased pain scores which persist until the end of the experiment. Pain behaviour in both phases of the formalin test was dose-dependently inhibited by L-29 administration, but they maintained the biphasic pattern of pain behaviour. Both 10 mg/kg L-29 and 1 mg/kg L-29 significantly attenuated pain behaviour ($P < 0.05$ by one-way ANOVA plus Dunnett's test v control), but 0.1 mg/kg L-29 was not significantly different from control. Although the 10 mg/kg dose of L-29 was not significantly more antinociceptive than the 1 mg/kg dose, a trend can be seen in Figure 4A.

3.2 Effects of receptor antagonists on L-29-evoked analgesia

Figure 5A shows the time course of pain behaviour for the control group, 1 mg/kg L-29 group and the receptor antagonist groups, which were 1 mg/kg L-29 with prior administration of either 1 mg/kg SR1, 1 mg/kg SR2 or 10 mg/kg capsazepine. Figure 5B shows the mean pain scores for phase 1 and 5 phase 2 of the above groups. All groups show the biphasic response to formalin injection. We wanted to discover whether the receptor antagonists decreased the antinociceptive effect of the L-29, so we analysed the data against the L-29 group. In phase 1 of the formalin test, the only group that was significantly different (P<0.05 by one-way ANOVA with Dunnett's test 10 v 1 mg/kg L-29) to L-29 was the control group. In phase 2 however, SR1 reverses the antinociceptive effect of L-29 in phase 2 of the formalin test. SR2 and capsazepine do not significantly reverse the antinociceptive effect of L-29 in either phase.

15

4. Discussion

The results demonstrate that L-29 reduces pain behaviour in a dose-dependent manner in both phase 1 and phase 2 of the formalin model of 20 inflammatory pain in the rat. SR1, a selective CB1 receptor antagonist, significantly prevents this antinociceptive effect of L-29 in phase 2, whereas SR2 and capsazepine do not reverse this action. This suggests that the effects of L-29 are mediated by increased levels of endocannabinoids 25 binding to CB1 receptors. Surprisingly, although both phase 1 and phase 2 nociceptive behaviour was attenuated by L-29, only the antinociceptive effect in phase 2 was reversed by SR1.

In all animal behavioural tests environmental factors have a large influence, if not carefully controlled this can lead to bias. Novelty stress induced 30 analgesia is a recognised phenomenon in rats placed in new environments,

mediated by release of endogenous opiates (review Yamada and Nabeshima 1995). To minimise any such variation we kept to a strict protocol. All rats were 6-8 weeks old and arrived from the supplier at the animal house at least 3 days before the experiment started. They were housed in group 5 cages of 5 as rats become stressed when housed in isolation, and were handled regularly to reduce stress due to handling during the experiment. On the day of the experiment rats arrived at the lab at least 1 hour before experiments began and were acclimatised to the observation chamber for at least 30 min before starting the experiment. There is also evidence that 10 ambient temperature can affect the formalin test (Rosland 1991), so all experiments were carried out in the same climate controlled room. Site of injection can also lead to variation (Puig and Sorkin 1995), so all injections were given by same investigator and any rats without noticeable oedema and redness in the injected paw (indicating failure of the injection) were 15 excluded.

The protocol for administering the treatment drugs was decided by reference to previous work. Pharmacological data available for SR1, (Strangman et al 1998) showed that SR1 has a latency to effect of 20 approximately 15 min, so we decided to administer all antagonist drugs 15 min before starting experiment. Previous data is not available for L-29, so we based our decision on available PEA data, which is structurally similar to L-29. PEA has been given i.p. immediately prior to the formalin test (Jaggar et al 1998b) and has also been given i.p. 30 minutes prior to 25 administration of noxious substances in the writhing test (Calignano et al 2001). We therefore decided to give L-29 10 minutes before formalin administration, as agonists should generally be given after their antagonist. This is because an antagonist may not work to full effect if the agonist is already occupying the receptor site. Dose of formalin used is also important 30 (Lee et al 2000). Lower concentrations will produce smaller pain responses,

potentially making it difficult to detect an analgesic effect. If too high a concentration is used, the maximum pain behaviour level may be reached, making it difficult to detect a hyperalgesic effect. We used a median dose of 2.5% formalin, which has been used routinely in our laboratory.

5

There are many different animal models of pain. Differences between nociceptive assays include the stimulus aetiology, intensity, location and duration of the stimulus and the characteristics of the response. The formalin test is caused by a chemical/inflammatory stimulus given in a 10 subcutaneous location producing a moderate intensity stimulus with a tonic but limited duration. It produces an organised and integrated behavioural response, not simply a reflex action. It's main advantage over models that look at simple nociceptive stimuli, such as the tail-flick test, are that it mimics human clinical pain conditions, in which pain lasts for a longer 15 period of time and is not escapable. The immediate onset and limited duration are an advantage over the more prolonged time course of other inflammatory pain models, such as carrageenan. Another advantage is that the formalin test uses a freely moving animal which prevents the confounding factor of endogenous analgesia caused by the stress of 20 restraint. A disadvantage of the formalin test is that the biphasic nature of the response makes interpretation of results more difficult when pharmacokinetics are uncertain. Although the formalin test is a robust model of inflammatory pain, further work using different pain models is needed to confirm the results we obtained in this study.

25

SR1 and SR2 may be inverse agonists rather than pure antagonists at cannabinoid receptors (Bouaboula et al 1997). Inverse agonists activate the receptor to produce effects opposite to that of the agonist. There is evidence that SR1 may cause hyperalgesia at the CB₁ receptor, suggesting either 30 antagonism of an endogenous cannabinoid tone or an inverse agonist effect.

Calignano et al 1998 showed a hyperalgesic effect of SR1 in the formalin test, however others (Beaulieu et al 2000) have not replicated this effect. Studies demonstrating an increase in levels of endogenous cannabinoids following formalin injection (Walker et al 1999) provide evidence that SR1 5 causes a reversal of endogenous tone rather than an inverse agonist effect. There is also extensive evidence that SR1 has no effect in many other systems (for review see Martin and Lichtman 1998).

The VR1 receptor is a cation channel activated by noxious heat and 10 capsaicin, the pungent ingredient in chilli peppers, and mediates 'burning' pain sensation. AEA is an endogenous ligand of cannabinoid receptors, and there is also evidence that it is a full agonist at VR1 (Smart et al 2000), although in this study this action was not at physiological relevant concentrations. On the other hand, there is some evidence that the action of 15 anandamide at VR1 receptors can be potentiated in certain circumstances. Inhibiting hydrolysis of anandamide has been shown to enhance the potency of anandamide as a VR1 ligand by at least 5 times (De Petrocellis et al 2001a). Increased levels of PEA have also been shown to increase anandamide action at VR1 receptors (De Petrocellis et al 2001). Therefore, 20 in view of a possible effect at VR1 receptors, we used capsazepine, a potent and selective VR1 antagonist (Bevan et al 1992) to detect any possible effect.

FAAH has a wide substrate specificity and is capable of metabolising a 25 wide range of AEA analogues and other fatty acid amides such as PEA and oleamide. Several standard compounds are capable of blocking FAAH activity including phenylmethylsulfonyl fluoride (PMSF) (Compton and Martin 1997) and methylarachidonylfluorophosphonate (MAPH) (Martin et al 2000). However these cannot be used therapeutically as they are too 30 toxic, so various AEA analogues and other fatty acid derivatives have been

investigated for their ability to inhibit FAAH (Lambert et al 1999, Boger et al 2000). It is doubtful whether FAAH inhibition will have much analgesic effect in the normal state, as many FAAH inhibitors lack cannabimimetic effects in vivo, although mice lacking FAAH (FAAH^{-/-}) do have reduced 5 pain sensation (Cravatt et al 2001). FAAH inhibitors may well be more beneficial in inflammatory states as levels of AEA and PEA are increased in these circumstances, for instance in a mouse model of multiple sclerosis (Baker et al 2000) and increased brain levels have been detected in response to the formalin test (Walker et al 1999). In addition, AEA and PEA given 10 exogenously have been found to reduce inflammatory pain (Jaggar et al 1998a, Calignano et al 2001).

This study has demonstrated that the FAAH inhibitor L-29 may work primarily to increase the levels of endogenous cannabinoids available for 15 binding to CB₁ receptors, however further work is needed to confirm our results and extend the understanding of the mechanisms of action of L-29. Another possibility could be that L-29 produces analgesic effects due to actions on a novel non-CB₁/CB₂ receptor. It is widely believed that there is a novel cannabinoid receptor, but this hypothesis has not yet been proved by 20 cloning of the receptor. The most likely endogenous ligand for this putative receptor is PEA. This makes it less likely that L-29 is acting via this receptor, as PEA's effects are antagonised by CB₂ receptor antagonists, not CB₁ receptor antagonists. It would also be useful to confirm the action of L-29 in several diverse pain models. A model of reflex withdrawal such as the 25 tail-flick test (D'Amour and Smith 1941); a model of visceral pain, such as colorectal distension (Ness and Gebhart 1988) or inflammatory visceral pain produced by turpentine instillation into the urinary bladder (McMahon and Abel 1987) and perhaps a model of neuropathic pain such as L5 and L6 spinal nerve ligation (Kim and Chung 1992). Tests performed after L-29 30 administration to evaluate the tetrad of cannabimimetic effects: catalepsy,

hypolocomotion, analgesia (already done) and hypothermia would determine if there is a generalised rise in endogenous cannabinoids. Anandamide produces all these cannabimimetic effects when given exogenously, so if the main effect of L-29 is to increase endogenous 5 anandamide, as suggested by administration of SRI, then L-29 may also produce similar effects.

CB1 agonists are undoubtedly antinociceptive, both in a variety of animal models of pain (review Pertwee 2001) and in clinical pain states, but dose-limiting psychotropic side effects have restricted their use thus far. 10 Therefore new strategies for manipulating the endocannabinoid system are being developed (review Porter and Felder 2001). Partial agonists, receptor modulators which have no agonist activity but potentiate the response to agonists, reuptake inhibitors and FAAH inhibitors may have more potential 15 as therapeutic agents. In this study we have demonstrated an antinociceptive role for a FAAH inhibitor in a clinically relevant model of inflammatory pain.

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Appendix

Table 1

Group	First i.p Injection	Second i.p Injection
1	Vehicle	Vehicle
2	Vehicle	10 mg/kg L-29
3	Vehicle	1 mg/kg L-29
4	Vehicle	0.1 mg/kg L-29
5	1 mg/kg SR144528	1 mg/kg L-29
6	1 mg/kg SR141716A	1 mg/kg L-29
7	10 mg/kg capsazepine	1 mg/kg L-29

Claims

1. Use of a compound of formula I:

5 $RC(O)-NH-(CH_2)_n-CH=CH_2$ wherein R represents C_{1-20} alkyl, C_{2-20} alkenyl or C_{2-20} alkynyl; and
n is an integer from 0 to 3,

in the manufacture of a medicament for use in pain relief.

10

2. A compound of formula I as defined in claim 1 for use in medicine.

15 3. A method of providing pain relief to a patient comprising
administering to said patient an effective amount of a compound of
formula I, as defined in claim 1.20 4. Use according to claim 1 or method according to claim 3 wherein the
medicament further comprises one or more analgesics or the patient
is administered said further one or more analgesics.

25

5. The use, compound or method of any of the previous claims wherein,
in the compound of formula I, R represents C_{10-20} alkyl, C_{10-20} alkenyl
or C_{10-20} alkynyl.25 6. The use, compound or method of any of claims 1 to 4 wherein, in the
compound of formula I, R represents C_{10-20} n-alkyl, C_{10-20} mono-
alkenyl or C_{10-20} mono-alkynyl.

7. The use, compound or method of any of claims 1 to 4 wherein, in the compound of formula I, R represents C_{10-20} *n*-alkyl, C_{10-20} mono-*n*-alkenyl or C_{10-20} mono-*n*-alkynyl.
- 5 8. The use, compound or method of any of claims 1 to 4 wherein, in the compound of formula I, R represents C_{11-19} *n*-alkyl, or C_{11-19} mono-*n*-alkenyl.
9. The use, compound or method of any of claims 1 to 4 wherein, in the compound of formula I, R represents C_{11-18} *n*-alkyl, or C_{11-18} mono-*n*-alkenyl.
- 10 10. The use, compound or method of any of claims 1 to 4 wherein, in the compound of formula I, the alkenyl or alkynyl groups have no more than 3 C-C double or triple bonds, respectively.
11. The use, compound or method of any of claims 1 to 5 wherein the compound is not *N*-(2-propenyl) - 5,8,11,14-eicosatetraenamide.
- 20 12. The use, compound or method of any of the previous claims wherein, in the compound of formula I, n represents 0 or 1.
13. The use, compound or method of claim 12 wherein, in the compound of formula I, n represents 1
- 25 14. The use, compound or method of any of claims 1 to 4 wherein the compound is *N*-(2-propenyl) hexadecanamide, *N*-(2-propenyl) cis-9-octadecenamide, *N*-(2-propenyl) cis-9-hexadecenamide, *N*-(2-propenyl) tetradecanamide, *N*-(2-propenyl) cis-9-tetradecenamide, *N*-(2-propenyl) octadecanamide, *N*-(2-propenyl) trans-9-
- 30

octadecenamide, *N*-(2-propenyl) dodecanamide, or *N*-(2-propenyl) *cis*-5-dodecenamide.

15. A use compound or method of any of the previous claims wherein
5 the compound is *N*-(2-propenyl) hexadecanamide.
16. A pharmaceutical composition comprising a compound as defined in
relation to any of the previous claims and one or more analgesics and
a pharmaceutically acceptable excipient.
10
17. A kit of parts comprising:
 - (a) a compound as defined in relation to any of the previous claims;
and,
 - (b) one or more analgesics; and,
 - (c) a pharmaceutically acceptable excipient
15
18. A use or method according to claim 4 or pharmaceutical composition
according to claim 16 or a kit of parts according to claim 17 wherein
20 the analgesic is an opioid, a non-steroidal anti-inflammatory drug, a
local anaesthetic, a NMDA receptor antagonist, a cannabinoid, an
antidepressant, and/or an anticonvulsant.
25

Abstract

Use of a compound of formula I: $RC(O)-NH-(CH_2)_n-CH=CH_2$, wherein R represents C_{1-20} alkyl, C_{2-20} alkenyl or C_{2-20} alkynyl; and n is an integer from 5 0 to 3, in the manufacture of a medicament for use in pain relief. A compound of formula I for use in medicine. A method of providing pain relief to a patient comprising administering to said patient an effective amount of a compound of formula I. A preferred compound is *N*-(2-propenyl) hexadecanamide.

10

Figure 1.



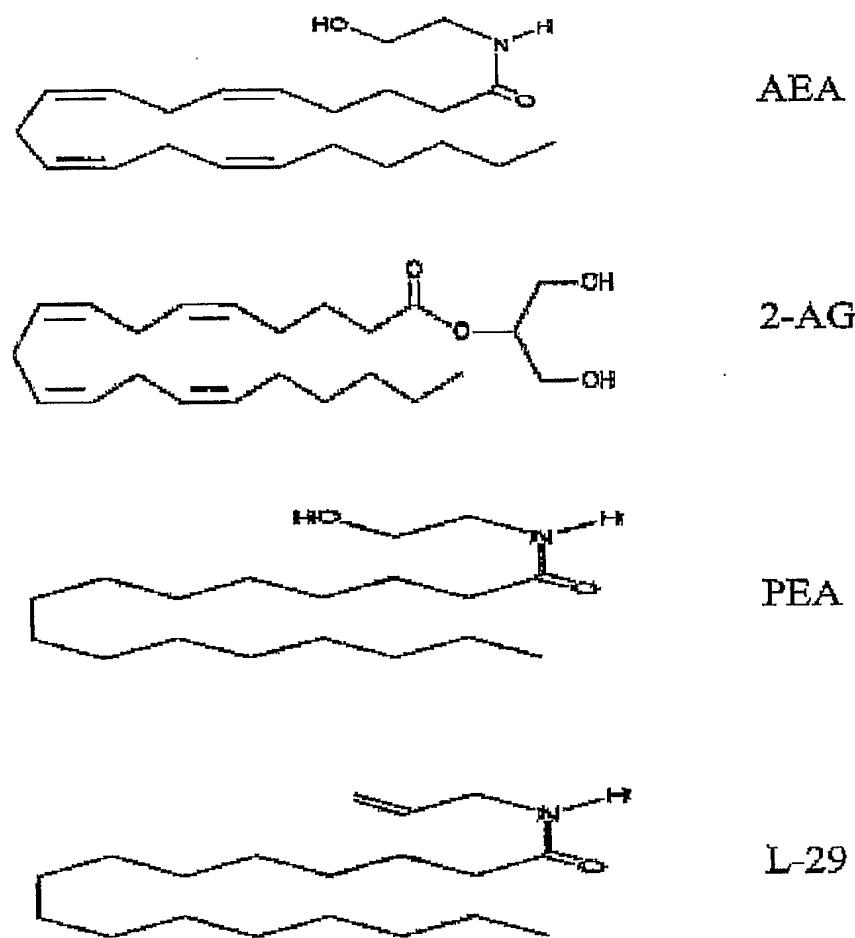
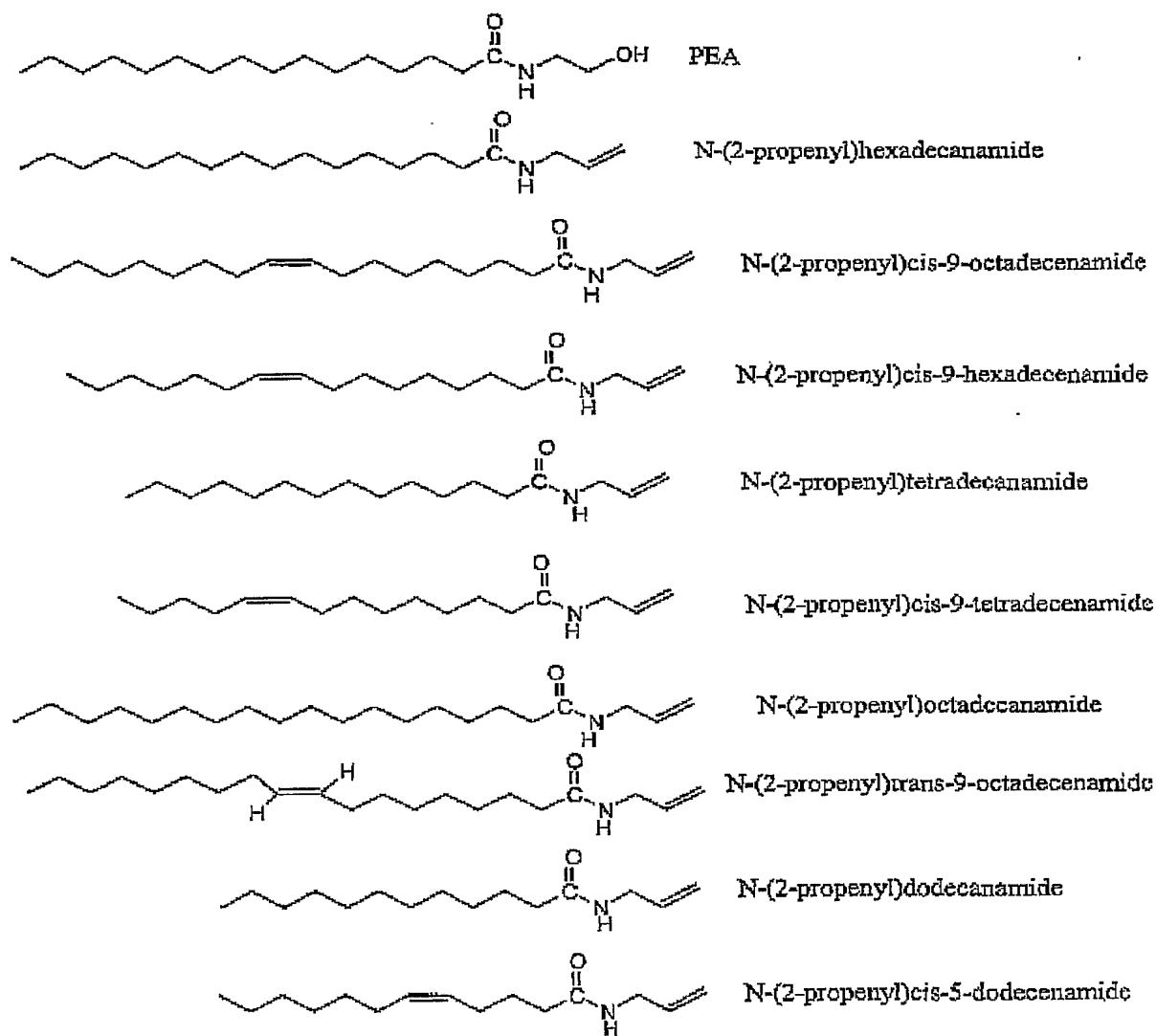
Figure 1**1 /8**

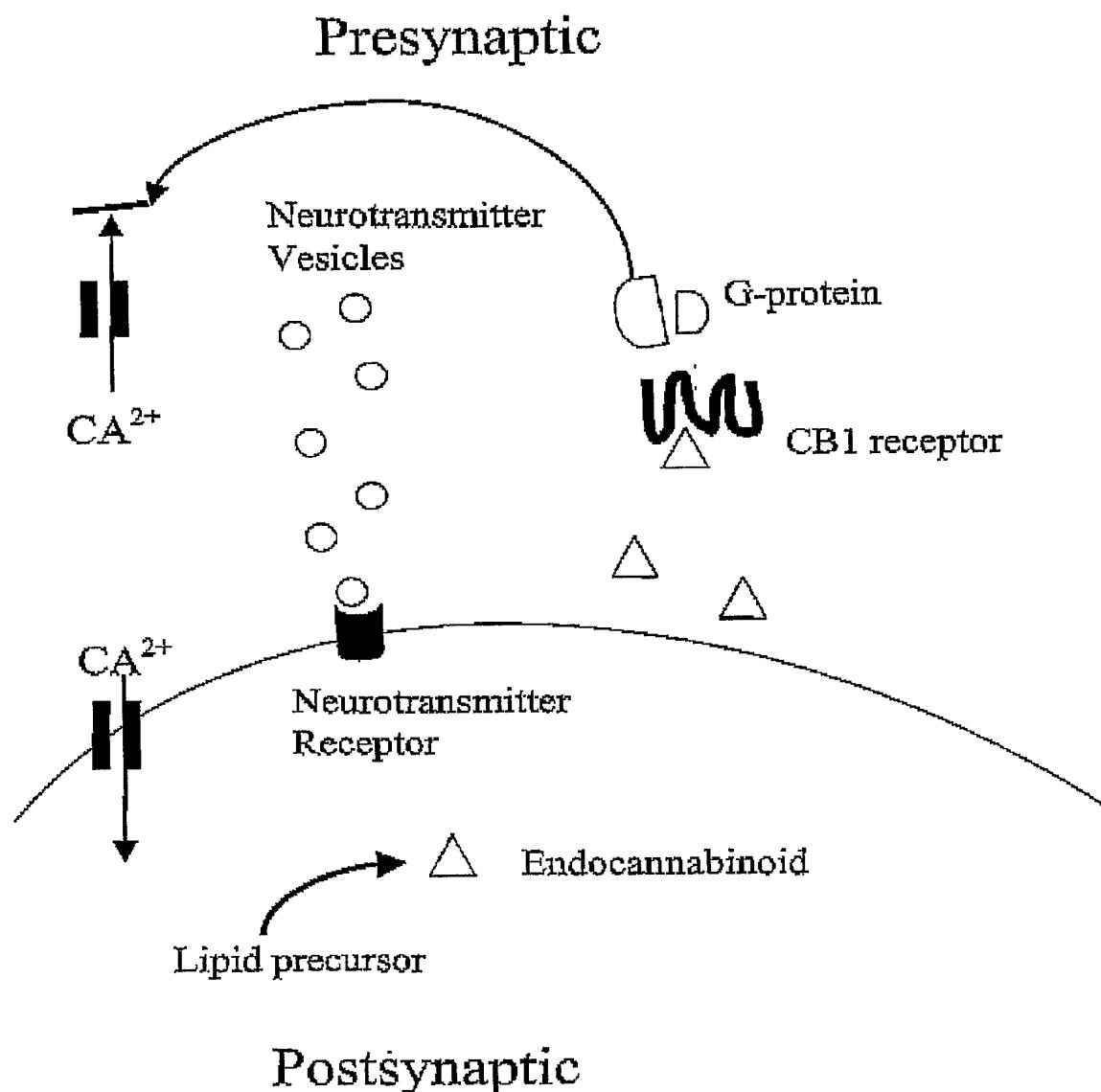


Figure 1B



2 /8



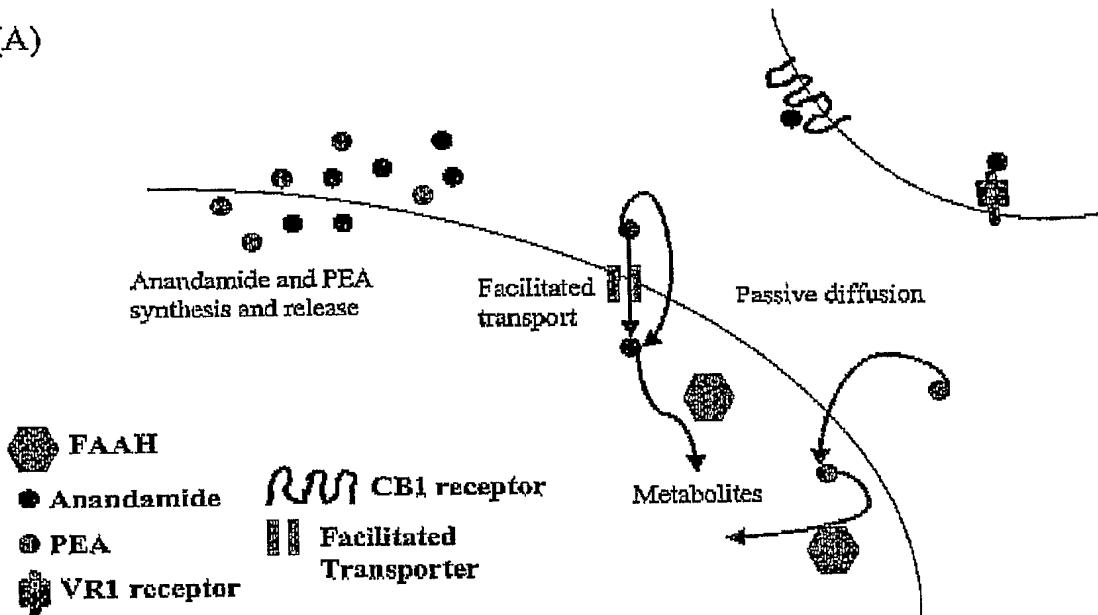
Figure 2

3 /8



Figure 3

(A)



(B)

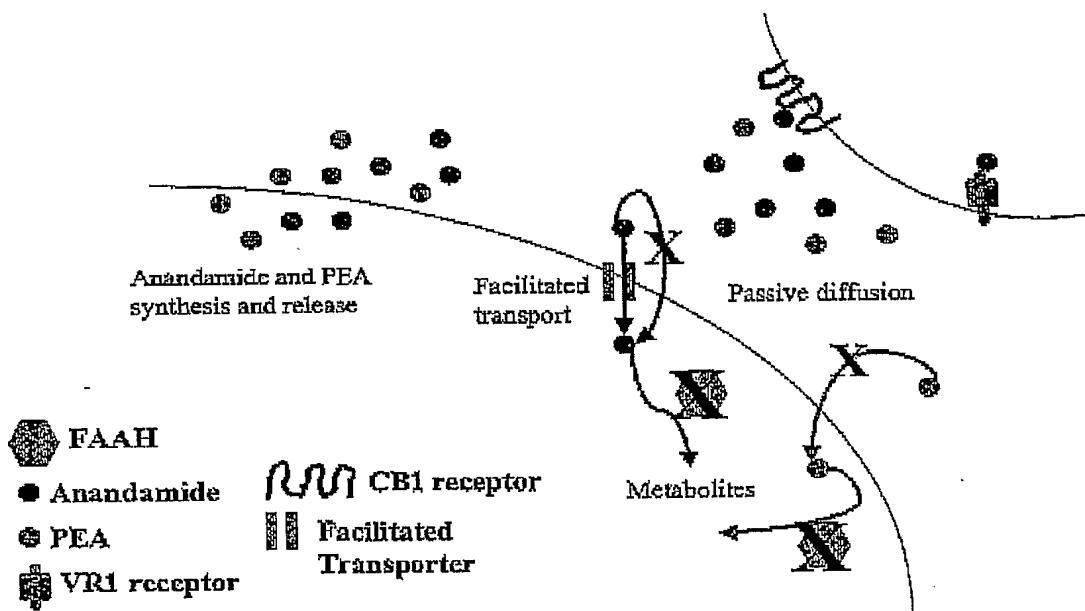
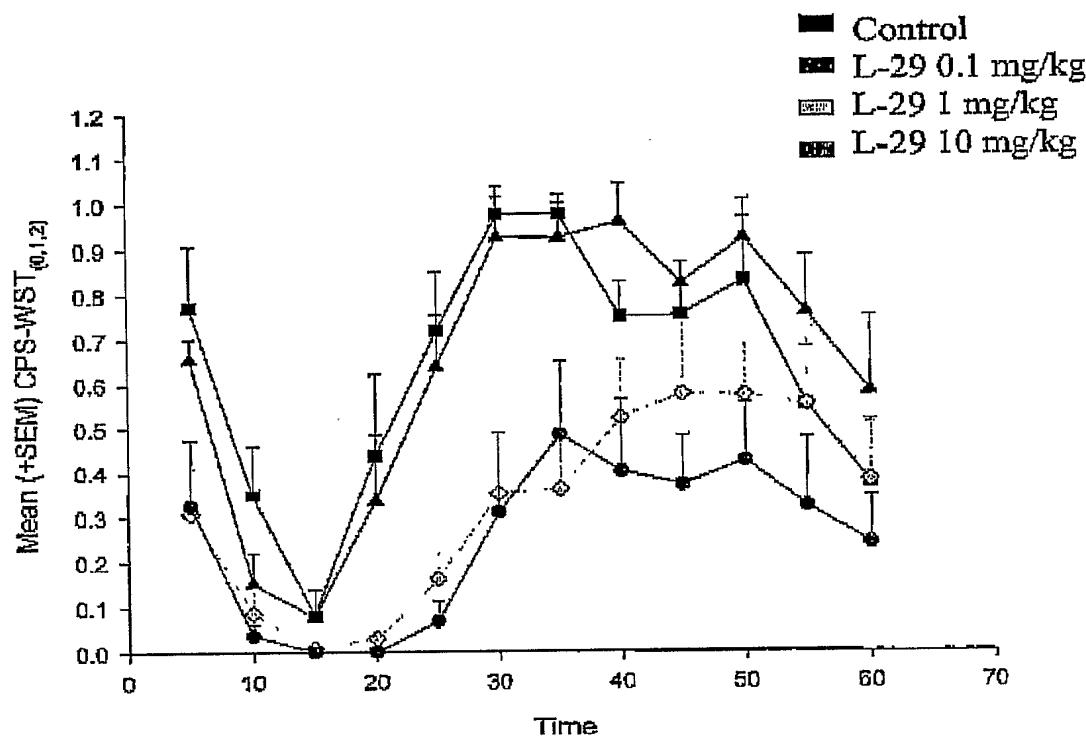


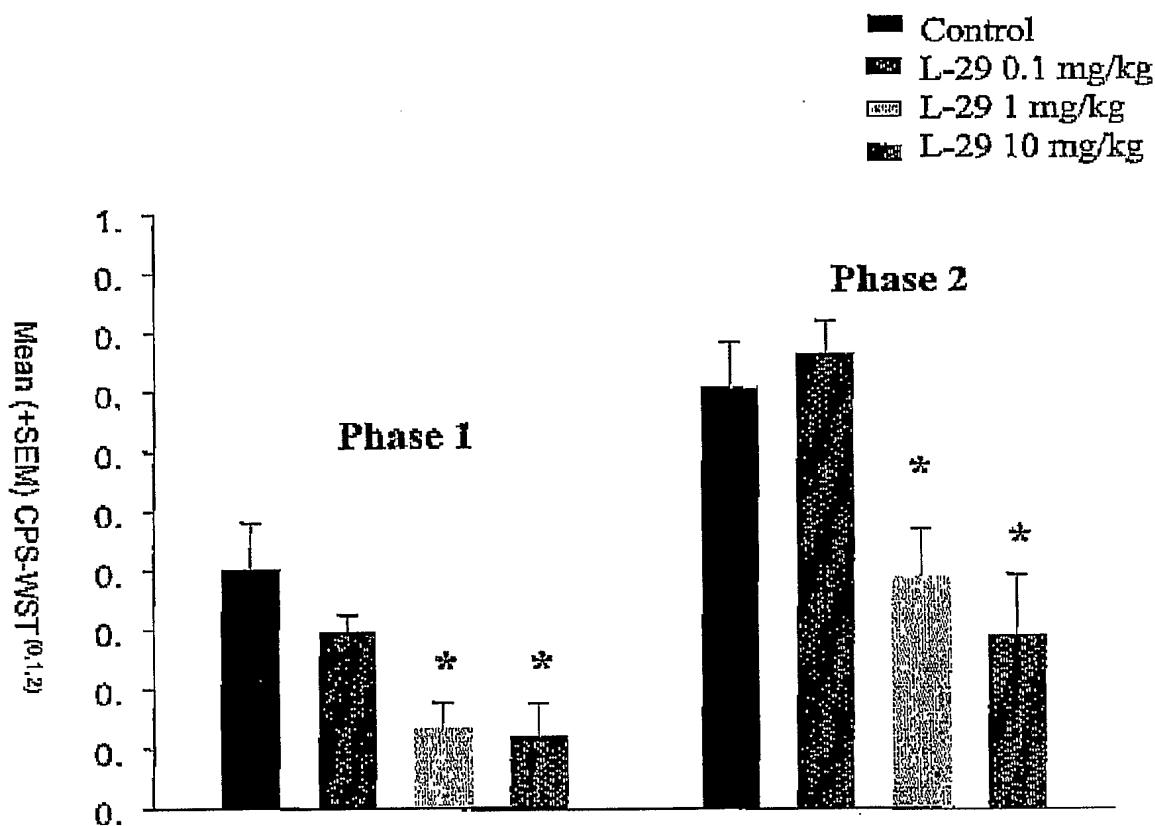


Figure 4A



5 /8

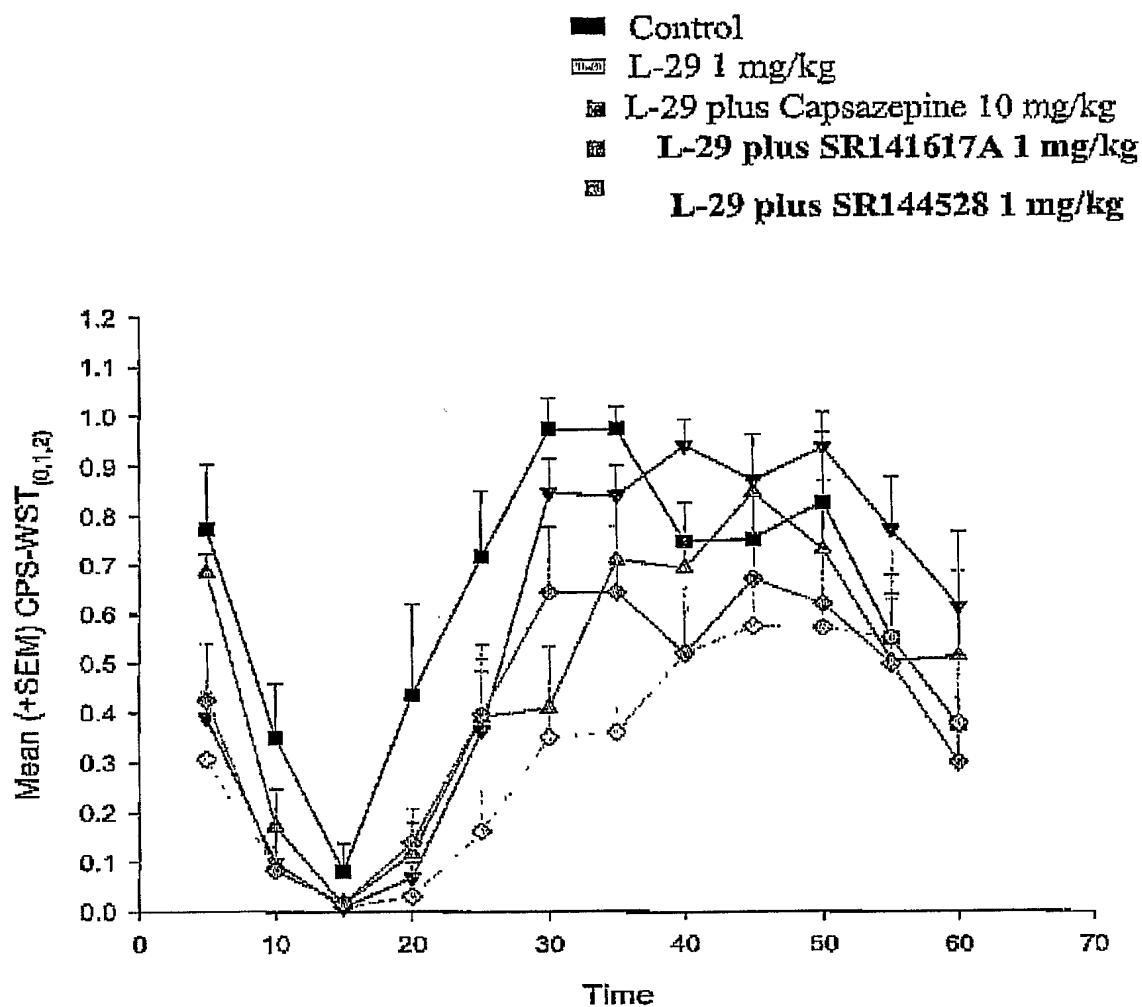
Figure 4B



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Figure 5A



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Figure 5B

